

Applicant : Imperial Cancer Research Technology Ltd.
 Serial No. :
 Filed : HEREWITH
 Page : 2

Attorney's Docket No.: 12795-009US1

than 32 amino acids in length and wherein the interacting polypeptide is not a kininogen or fragment thereof; or the G glycoprotein of human respiratory syncytial virus or fragment thereof; or a fibroblast growth factor receptor (FGFR); or the peptide SKPTTKQRQNKPPNKP.

52. An interacting polypeptide capable of interacting with a Smad polypeptide wherein the interacting polypeptide comprises a Smad Interaction Motif (SIM), for example the amino acid sequence PP(T/N)K or three out of four residues thereof, and is not full-length Xenopus or human FAST1 or a fragment thereof, mouse FAST2, Xenopus Milk, Xenopus Mixer, Xenopus Bix3 or Bix2 and wherein the interacting polypeptide is not a kininogen or fragment thereof; or the G glycoprotein of human respiratory syncytial virus or fragment thereof; or a fibroblast growth factor receptor (FGFR); or the peptide SKPTTKQRQNKPPNKP.

53. An interacting polypeptide capable of interacting with a Smad polypeptide wherein the interacting polypeptide comprises a Smad Interaction Motif (SIM), is less than 32 amino acids in length and is a transcription factor or fragment thereof.

54. An interacting polypeptide capable of interacting with a Smad polypeptide wherein the interacting polypeptide comprises the amino acid sequence PP(T/N)K, is less than 32 amino acids in length and is a transcription factor or fragment thereof.

55. An interacting polypeptide capable of interacting with a Smad polypeptide wherein the interacting polypeptide comprises a Smad Interaction Motif (SIM), for example the amino acid sequence PP(T/N)K or three out of four residues thereof, and is not full-length Xenopus or human FAST1 or a fragment thereof, mouse FAST2, Xenopus Milk, Xenopus Mixer, Xenopus Bix3 or Bix2 and is a transcription factor or fragment thereof.

56. The interacting polypeptide of claim 50, 52, 53, or 55 wherein the SIM comprises at least 8, 9 or 10 of the specified residues (ie not residues designated by an X) of the amino acid sequence D/E-Hyd-(X)_n-P-P-(N/T)-K-(T/S)-(I/V)-(X)_m-(D/E)-(M/V/I)-(X)_k-P

Applicant : Imperial Cancer Research Technology Ltd.
 Serial No. :
 Filed : HEREWITH
 Page : 3

Attorney's Docket No.: 12795-009US1

wherein m= 0 to 7; k= 0 to 8 or 12; n = 0 to 15 or 18.

57. The interacting polypeptide of claims 52 or 55 wherein the interacting polypeptide is less than 100 amino acids in length.

58. The interacting polypeptide of claim 57 wherein the polypeptide is between 4 and about 30 or 35 amino acids in length.

59. The interacting polypeptide of any one of claims 50-52 wherein the Smad polypeptide is Smad2 or Smad3.

60. The interacting polypeptide of any one of claims 50-55 wherein the interacting polypeptide is a transcription factor or a fragment thereof.

61. The polypeptide of any one of claims 50-55 wherein an acidic amino acid residue is present at a position from 3 to 10 residues C-terminal of the amino acid sequence PP(T/N)K or amino acid sequence corresponding to the PP(T/N)K motif and/or a proline residue is present at a position from 5 to 20 residues C-terminal of the amino acid sequence PP(T/N)K K or amino acid sequence corresponding to the PP(T/N)K motif.

62. The polypeptide of any one of claims 50-55 comprising the amino acid sequence PPNKTITPDMNVRIPPI or PPNKTITPDMNTIIPQI or PPNKSVFDVLTSHPGD or PPNKSIYDVWVSHPRD or PPNKSIYDVWVSHPRD or PPNKTVFDIPVYTGHGP or PPNKTITPDMNTIIPQI or PPNKTIGPEMKVVIPPL or PPNKSSKRGNTPPW or LLMDFNFPNKTITPDMNVRIPPI or HSNLMMDFPPNKTITPDMNTIIPQI or LDNMLRAMPPNKSVDVLTSHPGD or LDSLFQGVPPNKSIDVWVSHPRD or LDALFQGVPPNKSIDVWVSHPRD or LKNAPSDFPNKTIVFDIPVYTGHGP or HSNLVMEFPNKTITPDMNTIIPQI or LVEYDNFPNKTIGPEMKVVIPPL or ITSDAYSDESCPPNKS KRGNTPPW.

Applicant : Imperial Cancer Research Technology Ltd.
 Serial No. :
 Filed : HEREWITH
 Page : 4

Attorney's Docket No.: 12795-009US1

63. A polypeptide consisting of the amino acid sequence PPNKTITPDMNVRIPPI or PPNKTITPDMNTIIPQI or PPNKSVFDVLTSHPGD or PPNKSIYDVWVSHPRD or PPNKSIYDVWVSHPRD or PPNKTVFDIPVYTGHGP or PPNKTITPDMNTIIPQI or PPNKTIGPEMKVVIPPL or PPNKSSKRGNTPPW or LLMDFNFFPPNKTITPDMNVRIPPI or HSNLMMDFFPPNKTITPDMNTIIPQI or LDNMLRAMPPNKSVDVLTSHPGD or LDSLFQGVPPNKSIVDVWVSHPRD or LDALFQGVPPNKSIVDVWVSHPRD or LKNAPSDFFPPNKTIVFDIPVYTGHGP or HSNLVMEFFPPNKTITPDMNTIIPQI or LVEYDNFFPPNKTIGPEMKVVIPPL or ITSDAYS DSCPPPNKSSKRGNTPPW.

64. The interacting polypeptide of claims 50-55 comprising the amino acid sequence of residues 283 to 307 of Mixer.

65. The interacting polypeptide of claims 50-55 wherein the said polypeptide is a peptidomimetic compound.

66. A molecule comprising an interacting polypeptide as defined in any one of claims 50-55 and a further portion, wherein the said molecule is not full-length Xenopus or human FAST1 or a fragment thereof, mouse FAST2, Xenopus Milk, Xenopus Mixer or Xenopus Bix2.

67. A molecule according to claim 66 wherein the molecule is Biotin.Aminohexanoicacid-RQIKIWFQNRRMKWKKLLMDFNFFPPNKTITPDMNVRIPPI or 5-FAM-AMINOHEXANOICACID-RQIKIWFQNRRMKWKKPEVKNAPKDFPPNKTIVFDIPVYTGHGPFLA

68. A nucleic acid encoding the polypeptide of any one of claims 50-55 and 63.

69. A nucleic acid complementary to the nucleic acid encoding the polypeptide of claim 68.

Applicant : Imperial Cancer Research Technology Ltd.
Serial No. :
Filed : HEREWITH
Page : 5

Attorney's Docket No.: 12795-009US1

70. An antibody capable of selectively binding with a polypeptide according to any one of claims 50-55 and 63.

71. A method of identifying a polypeptide that is capable of interacting with a Smad polypeptide, comprising examining the sequence of a polypeptide and determining that the polypeptide comprises a Smad Interaction Motif (SIM), for example the amino acid sequence PP(T/N)K or three out of four residues thereof.

72. The method of claim 71 comprising determining that the polypeptide comprises at least 8, 9 or 10 of the specified residues other than X of the amino acid sequence D/E-Hyd-(X)_n-P-P-(N/T)-K-(T/S)-(I/V)-(X)_m-(D/E)-(M/V/I)-(X)_k-P
wherein m= 0 to 7; k= 0 to 8 or 12; n = 0 to 15 or 18.

73. The method of claims 71 or 72 comprising determining that the polypeptide comprises the amino acid sequence PP(T/N)K.

74. The method of claims 71 or 72 further comprising determining that an acid amino acid residue is present at a position from 3 to 10 residues C-terminal of the amino acid sequence PP(T/N)K or amino acid sequence corresponding to the PP(T/N)K motif, and/or a proline residue is present at a position from 5 to 20 residues C-terminal of the amino acid sequence PP(T/N)K or amino acid sequence corresponding to the PP(T/N)K motif.

75. A method of identifying a compound capable of disrupting or preventing the interaction between a Smad polypeptide and a target polypeptide that is (1) a transcription factor capable of interacting with the said Smad polypeptide and/or (2) a polypeptide capable of interacting with the said Smad polypeptide, the interaction requiring α -helix2 of the said Smad polypeptide or (3) a polypeptide comprising the amino acid sequence PP(T/N)K, the method comprising measuring the ability of the compound to disrupt or prevent the interaction between the Smad polypeptide and a polypeptide or molecule according to any one of claims 50-55 and 63.

Applicant : Imperial Cancer Research Technology Ltd.
Serial No. :
Filed : HEREWITH
Page : 6

Attorney's Docket No.: 12795-009US1

76. A compound identified by or identifiable by the method of claim 73 or claim 74.
77. A kit comprising a Smad polypeptide and a polypeptide or molecule according to any one of claims 50-55, 63 and 66.
78. A method of disrupting or preventing the interaction between a Smad polypeptide and a target polypeptide that is (1) a transcription factor capable of interacting with the said Smad polypeptide and/or (2) a polypeptide capable of interacting with the said Smad polypeptide, the interaction requiring a-helix2 of the said Smad polypeptide, the method comprising exposing the Smad polypeptide to a polypeptide or molecule according to any one of claims 50-55 and 63.
79. A method of disrupting or preventing the interaction between a Smad polypeptide and a polypeptide comprising the amino acid sequence PP(T/N)K wherein the Smad polypeptide is exposed to a polypeptide or molecule according to any one of claims 50-55 and 63.
80. The method of claim 78 wherein the Smad polypeptide is Smad2 or Smad3.
81. A composition comprising the polypeptide according to any one of claims 50-55 and a pharmaceutically acceptable carrier.
82. A method of modulating activin or TGFb signalling in a cell in vitro comprising exposing the cell to the polypeptide of any one of claims 50-55.
83. A method of modulating activin or TGFb signalling in a cell in vivo comprising exposing the cell to the polypeptide of any one of claims 50-53.
84. The method of claim 83 wherein the cell is a late stage tumor cell.

Applicant : Imperial Cancer Research Technology Ltd.
Serial No. :
Filed : HEREWITH
Page : 7

Attorney's Docket No.: 12795-009US1

85. A method for modulating activin or TGF β signaling in a patient comprising administering the polypeptide of any of claims 50-55.

86. A method for treating cancer comprising administering the polypeptide of any one of claims 50-55.

87. A method for treating a patient in need of reducing extracellular matrix deposition, encouraging tissue repair and/or regeneration, tissue remodelling or healing of a wound, injury or surgery, or reducing scar tissue formation arising from injury to the brain comprising administering the polypeptide of any of claims 50-55.

88. A method for treating a patient with or at risk of end-stage organ failure, pathologic extracellular matrix accumulation, a fibrotic condition, disease states associated with immunosuppression (such as different forms of malignancy, chronic degenerative diseases, and AIDS), diabetic nephropathy, tumour growth, kidney damage (for example obstructive neuropathy, IgA nephropathy or non-inflammatory renal disease) or renal fibrosis comprising administering the polypeptide of any of claims 50-55.

89. A substantially pure complex comprising: (1) a Smad2 or Smad3 polypeptide, (2) a Smad4 polypeptide and (3) a Mixer and/or Milk and/or Bix2/3 and/or FAST3 polypeptide.

90. A preparation comprising: (1) Smad2 or Smad3 polypeptide, (2) a Smad4 polypeptide and (3) a Mixer and/or Milk and/or Bix2/3 and/or FAST3 polypeptide (in the form of a complex or otherwise) when combined with other components ex vivo, said other components not being all of the components found in the cell in which said (1) Smad2 or Smad3 polypeptide, (2) a Smad4 polypeptide and (3) a Mixer and/or Milk and/or Bix2/3 and/or FAST3 polypeptide (in the form of a complex or otherwise) are naturally found.

91. A cell comprising: 1) a recombinant polynucleotide suitable for expressing a transcription factor that is capable of interacting with a Smad polypeptide and 2) a recombinant

Applicant : Imperial Cancer Research Technology Ltd.
Serial No. :
Filed : HEREWITH
Page : 8

Attorney's Docket No.: 12795-009US1

polynucleotide comprising a reporter gene driven by a promoter with a binding site for the said transcription factor.

92. A stable cell line cell comprising a reporter gene driven by a promoter with one or more binding sites for an activated Smad, wherein the Smad is activated in the cell by exposure of the cell to TGFb.

93. The cell according to claims 91 or 92 wherein the reporter gene expresses luciferase, secreted alkaline phosphatase (SEAP), CAT or a green fluorescent protein (GFP).

94. A method of identifying a compound capable of modulating TGFb-dependent transcription wherein the effect of the compound on expression of the reporter gene in a cell according to claims 91 or 92 is measured, following treatment of the cell with TGFb.

95. A method of identifying a compound capable of modulating TGFb-dependent transcription wherein the effect of the compound on TGFb-signalling-dependent invasive behaviour of a stably-transformed cell line cell, for example in collagen gels, is measured and a compound that reduces invasive behaviour is selected.

96. The method of claim 95 wherein the stably-transformed cell line is a MDCK cell line that is capable of expressing recombinant active Raf-1.--